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(57) Abstract

The present invention relates to the isolation, identification and characterization of a newly identified human gene responsible for disorders relating to ataxia, as well as the diagnosis and therapy of such disorders.

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GENE FOR ATAXIA

The present invention relates to the isolation, identification and characterization of newly identified human gene responsible for disorders relating to ataxia, as well as the diagnosis and therapy of such disorders.

Ataxia in general describes the inability of a patient to properly coordinate his or her movements and is caused by neuronal defects. The autosomal dominant forms mostly represent neurodegenerative disorders and are characterized by the selective loss of neurons within the brainstem and spinocerebellar tracts. Recessive forms of ataxia are often based on defects in neuronal cell communication, such as defective ion channels (Doyle and Stubbs, 1998).

Up to now 8 types of dominantly inherited ataxia have been genetically defined: Spinocerebellar Ataxia type 1-7 (SCA1-7) and Dentato-Rubral Pallido-Luysian Atrophy (DRPLA). Recent years have seen an impressive genetic contribution. The first gene for hereditary ataxia, SCAl, was discovered in 1993, and the gene of SCA7 was identified in 1996. Genetic mutations of SCA1, 2, 3, 6 and 7, have now been identified. For SCA4 and SCA5 the responsible genes have not been identified yet. For the SCA's with a known genetic mutation, individual genetic diagnosis and presymptomatic testing is possible.

Thus far, all known mutations for the different SCA's and for DRPLA consist of an unstable expansion of a CAG repeat in one of the gene's exons (reading frames). ‚CAG' stands for cytosine, adenine and guanine, the names of separate DNA molecules, which form a ‚triplet'. Dominant hereditary ataxia's are not the only disorders caused by an expanded triplet repeat. Among the disorders caused by an expanded triplet repeat are Huntington's disease, Friedreich's ataxia and Myotonic dystrophy.

In the SCA's, DRPLA, and in some other inherited diseases like Huntington's disease, a normally present CAG triplet repeat is expanded and has become instable. Translated into a protein, this expanded CAG triplet repeat results in a expanded tract of identical glutamine amino acids (a polyglutamine tract). Once the gene has been identified, the gene-products, proteins, can be studied. With one exception, SCA6, the proteins are previously unknown, novel proteins, with unknown functions. For SCA, the novel proteins are named ‚ataxin' with the corresponding SCA number, e.g. ataxin-l. Similarly

for DRPLA the protein is called 'atrophin', for Huntington's disease the protein is called ‚huntingtin' and for Friedreich´s ataxia, the protein is called ‚frataxin'.

SCA6 is the only Spinocerebellar Ataxia in which the function of the gene and protein is known. The protein in SCA6 is part of a voltage gated calcium channel in the cellular membrane, which plays a role in the cellular excitation. Other mutations in the gene for SCA6 can cause two other (allelic) disorders: hemiplegic migraine and episodic ataxia type II.

Combining the clinical information in the different Spinocerebellar Ataxia´s, some correlations become apparent. Within groups of SCA1, SCA2, SCA3, and SCA7 patients, the length of the CAG repeat shows a strong inverse correlation with the age at onset of symptoms, and also with progression of the disease. So, people with a later onset of symptoms usually have a smaller expansion of the CAG repeat. However, at any repeat length there is a rather wide variation in age at onset, of some twenty years or so. Thus, in a single individual the repeat length does not enable prediction of the age at onset or progression. Although essentially similar during an individual's lifetime, the CAG repeat is not stable during parent to child transmission, and may thus be different in parents and children. Especially in paternal transmissions, the expansion often increases. This in part explains anticipation, the phenomenon that the mean age at onset is earlier in next generations. In maternal transmission the repeat expansion is usually more stable.

Clinical manifestations vary considerably within each genetically defined type, and with exception of SCA7 and SCA6, only few global characteristics may be discerned for the SCA's, precluding an individual clinical diagnosis.

Research on histological and pathological aspects has focused mainly on the mechanism which causes cell death and ataxia. First, the presence of ataxin has been studied. As SCA's are dominantly inherited disorders, both a normal and a mutated SCA gene are present, and so both normal and mutated ataxin are expressed. It must be realised that genes are not active all the time, and that genes are turned on or off by signals according to cellular activity and specialisation. In the case of SCA1 and SCA2 for instance, it appears that ataxin-1 and ataxin-2 are present in many tissues and cells, and that the levels of ataxin do not correlate well with the presence of localised neurological cell death.

To explain the discrepancy between the presence of (mutated) ataxin and cell degeneration, interaction with other, possibly cell specific proteins has been suggested, and indeed, in Huntington's disease and various SCA's, aggregation with proteins has been demonstrated. Also, a tendency has been demonstrated for mutated ataxins to (self)aggregate, and proteins containing an expanded polyglutamine stretch have been found te be toxic for cultured cells.

Last year it was reported that in Huntington's disease and in SCA3 or Machado-Joseph Disease, certain brain cells showed inclusion bodies within the cell's nucleus. These inclusion bodies contained partially degraded molecules of mutated huntingtin and ataxin. Normally, huntingtin and ataxin are present in the cytoplasm of these cells, that is the fluid surrounding the nucleus, hut not within the nucleus. The presence of intranuclear inclusion bodies seemed to correlate with degeneration of these neurons. As these inclusion bodies also contained a protein degrading molecule, named ubiquitin, these inclusion bodies seem to indicate that the protein aggregate which they contain is resistant to degradation.

Friedreich's ataxia is the most frequent recessive ataxia occurring among white populations. But there are other recessive ataxias as well. They inelude: a) Ataxia-Telangiectasia (AT). This is an early onset ataxia with dilated veinules and telangiectasia. AT as it is known is almost as frequent as Freidreich's ataxia. b) Familial Vitamin E Deficiency, of which there are two forms, with or without fat malabsorption. The form without fat malabsorption is more common in the North African population, but rare everywhere else. Vitamin E supplementation is an effective treatment. Further known ataxias are c) Refsum Disease (ataxia with neuropathy and retinitis). d) Infantile Onset Spinocerebellar Ataxia, or IOSCA, a Finnish disease unknown in other world populations. e) Spastic Ataxia of Charlevoix-Saguenay. This is found mostly in Quebec, Canada. There exist further rare ataxias that don't have a name because they are not well charactenised. In two copies of the ataxia defect or mutation, one received from each parent. The parents of the patient are not affected because they carry only one copy. For all recessive diseases, the frequency of healthy carriers is much higher than the frequeney of patients, because the risk that two carriers of the same ataxia mutation marry is relatively low. For example, for Friedreich's ataxia, the frequency of healthy carriers is 1 in 120 while the frequeney of patients is 1 in 40,000. Almost all Friedreich's ataxia carriers and patients have the same ancestor (who lived most likely more than 10,000 years ago). This explains why Friedreich ataxia is more prevalent in

white populations and is almost non-existent among Japanese and black African populations.

The genetic defect in the gene leads to a lower expression of the protein called frataxin which is expressed about 10 to 20 times less compared to the expression in healthy persons. The important point is that there is still a little bit of frataxin made. This distinguishes the expansion mutation from other mutations, referred to as truncating mutations and frequently found in other recessive diseases which completely alter the production of the protein. Frataxin is a protein of the mitochondria, the structures of a cell where its fuel is produced from food. This energy conversion requires electron transport with iron containing proteins. Frataxin is present in tissues that are rich in mitochondria, which include the tissues that are affected in patients (dorsal root ganglia of the spinal cord, heart). Frataxin is present in every living organism, including fungi and yeast, because they also contain mitochondria. In yeast complete absence of frataxin causes iron accumulation within the mitochondria. In Friedreich's ataxia patients, a similar defect is also likely to apply, but to a lesser extent since there is still some residual frataxin present. Evidence for this are iron deposits and defect of some iron containing proteins in heart of patients. Mitochondrial iron accumulation seems therefore to be the culprit, since excess of iron is well known to stimulate the production of toxic compounds called free radicals on reactive oxygen species.

The present invention relates to the isolation and characterization of genes involved in constitution and regulation of the vertebrate nervous system, which is a major step towards elucidating the complex interactions required for an intact nervous system. The gene was identified via a positional cloning approach in the course of which the cytogenetic breakpoints of a patient suffering from severe cerebellar ataxia had been studied.

In order to investigate the breakpoint on the long arm of the X-chromosome, several YAC, PAC and cosmid clones (Vetrie et al., 1994) were chosen covering the region of CEPHy904G09223. DNA of these clones was used for FisH-analysis on metaphase spreads of the patient. It could be demonstrated that the PAC clone 1055C14 covers the breakpoint. The cosmids in close vicinity to the breakpoint-spanning PAC had already been sequenced by the Sanger Center in Cambridge.

Sequence analysis of Cosmid 9N5 allowed the design of appropriate primers for certain interesting regions. This allowed the amplifcation of parts of the potential new gene using a fetal brain cDNA library. The first seven exons of the gene could be identified. In order to obtain a full length cDNA clone, RACE experiments were performed and the resulting fragments were subcloned into a TOPO-vector (Invitrogen) and sequenced.

The complete cDNA has a coding region of 1251 bp encoding for 417 amino acids and a 3' untranslated region of 348 bp. The gene consists of nine exons ranging in size from 68 bp to 224 bp and is orientated from telomere (5'end) to centromere (3' end) (fig. 3). Exon 8 and 9 as well as the 3'UTR represent novel sequence data. The sequence as well as the translation into the potential protein are given in SEQ ID No. 1 and SEQ ID No. 2.

With respect to patient T.G., it was important to show whether the chromosomal breakpoint had affected this new gene. For this purpose all genomic fragments containing exons of the ataxia gene were isolated from PAC 1055C14, pooled and used for FisH-analysis on metaphase spreads of the patient's mother. The normal X-chromosome showed a signal in Xq22 plus a crosshybridization to Xq28. The rearranged X-chromosome showed a strong signal in Xp22, the crosshybridization signal and a weak signal in Xq22. This shows clearly that the breakpoint of the patient resides within the genomic locus of the ataxia gene.

To further verify this observation, Southern blot analysis was performed, using as a probe the entire ataxia cDNA. When using EcoRV as restriction enzyme, a band shift was observed in the patient, but not in healthy controls. This suggests, that indeed this novel gene is affected by the chromosomal breakage that has occured in the patient and might therefore contribute to the phenotype seen in the patient.

However, the gene responsible for the correct mediation of neuronal signalling can be used with respect to the diagnostic determination of other neurological diseases with yet unknown etiology, such as spastic paraplegia mutations in PLP, Pelizaeus-Merzbacher disease, diseases related to the vertebrate central nervous system and other various neurological diseases of yet unkown etiology.

The isolated genomic DNA or fragments thereof can be used for pharmaceutical purposes or as diagnostic tools or reagents for identification or characterization of the genetic defect involved in the disorders and diseases mentioned above.

Subject of the present invention are further ataxia proteins which are expressed after transcription of the ataxia gene into RNA or mRNA and which can be used in the therapeutic treatment of disorders related to mutations in said genes. The invention further relates to appropriate cDNA sequences which can be used for the preparation of recombinant proteins suitable for the treatment of such disorders.

Subject of the invention are further plasmid vectors for the expression of the DNA of these genes and appropriate cells containing such DNAs. It is a further subject of the present invention to provide means and methods for the genetic treatment of such disorders in the area of molecular medicine using an expression plasmid prepared by incorporating the nucleic acid molecules of this invention downstream from an expression promotor which effects expression in a mammalian host cell.

Brief Description of the SEQ ID:

SEQ ID NO. 1: Sequence of ataxia cDNA. Exon/Intron junctions are at the following positions: 72/73 (exon 1; size: 72 bp); 202/203 (exon 2; size: 130 bp); 270/271 (exon 3; size: 68 bp); 494/495 (exon 4; size: 224 bp); 575/576 (exon 5; size: 81 bp); 718/719 (exon 6; size: 143 bp); 933/934 (exon 7; size: 215 bp); 1083/1084 (exon 8; size: 150 bp); 1252 ATG stop codon (exon 9; size: 169 bp).

SEQ ID NO. 2: ataxia protein

SEQ ID NO. 3: exon 8 with neighbouring genomic sequences of the ataxia gene. Direction is from 5′ towards the 3′ end.

SEQ ID NO. 4: amino acid sequence for exon 8

SEQ ID NO. 5: exon 9 with neighbouring genomic sequences of the ataxia gene. Direction is from 5′ towards 3′ end.

SEQ ID NO. 6: part of the genomic sequence as published by the Sanger Center comprising exons 1 – 7. Exon/intron boundaries are at the following position of the sequence: exon1: 29850 – 29921 (72 bp); exon 2: 33026 – 33155 (130 bp); exon 3: 33445 – 33514 (68 bp); exon 4: 33752 – 33975 (224 bp); exon 5: 34115 – 34195 (81 bp); exon 6: 35760 – 35901 (143 bp); exon 7: 38782 – 38996 (215 bp). With respect to the published complementary strand, the exon/intron boundaries

(5'-boundary to 3'-boundary) are at the following positions of the sequence: exon 1: 9946 – 9875 (72 bp); exon 2: 6770 – 6641 (130 bp); exon 3: 6351 – 6282; (68 bp); exon 4: 6044 – 5821 (224 bp); exon 5: 5681 – 5601 (81 bp); exon 6: 4036 – 3895 (143 bp); exon 7: 1014 – 800 (215 bp).

Brief Description of the figures:

Fig. 1: Schematic presentation of the genomic organization of the gene. Exons 1 - 7 were already sequenced by genome-wide sequencing efforts. Exons 8 and 9 as well as the 3'UTR have been sequenced according to the present invention.

Since the target gene leading to ataxia was unknown prior to the present invention, the biological and clinical association of patients with this deletion could give insights to the function of this gene. In the present study, fluorescence in situ hybridization (FISH) was used to examine metaphase and interphase lymphocyte nuclei of patients.

Subject of the present invention are therefore DNA sequences or fragments thereof which are part of the genes responsible for ataxia. DNA sequences or fragments of this gene, as well as the respective full length DNA sequence of this gene can be transformed in an appropriate vector and transfected into cells. When such vectors are introduced into cells in an appropriate way as they are present in healthy humans, it is devisable to treat diseases of patients suffering from various types of ataxia by modern means of gene therapy. For example, ataxia can be treated by removing the respective mutated gene. It is also possible to stimulate the respective genes which compensate the action of the genes responsible for ataxia, i.e. by inserting DNA sequences before, after or within the ataxia gene in order to increase the expression of the healthy allels. By such modifications of the genes, the ataxia gene become activated or silent, respectively. This can be accomplished by inserting DNA sequences at appropriate sites within or adjacent to the gene, so that these inserted DNA sequences interfere with the ataxia gene and thereby activate or prevent their transcription. It is also devisable to insert a regulatory element (e.g. a promotor sequence) before said ataxia gene to stimulate the gene to become active. It is further devisable to stimulate the respective promotor sequence in order to overexpress the healthy functional allele. The modification of genes can be generally achieved by inserting exogenous DNA sequences into the ataxia gene via homologous recombination.

The DNA sequences according to the present invention can also be used for transformation of said sequences into animals, such as mammals, via an appropriate vector system. These transgenic animals can then be used for *in vivo* investigations for screening or identifying pharamceutical agents which are useful in the treatment of diseases involved with defects of the ataxia gene. If the animals positively respond to the administration of a candidate compound or agent, such agent or compound or derivatives thereof would be devisable as pharmaceutical agents. By appropriate means, the DNA sequences of the present invention can also be used in genetic experiments aiming at finding methods in order to compensate for the loss of genes responsible for ataxia (knock-out animals).

In a further object of this invention, the DNA sequences can also be used to be transformed into cells. These cells can be used for identifying pharmaceutical agents useful for the treatment of diseases involved with ataxia, or for screening of such compounds or library of compounds, especially using high-throuput screening systems. In an appropriate test system, variations in the phenotype or in the expression pattern of these cells can be determined, thereby allowing the identification of interesting candidate agents in the development of pharmaceutical drugs.

The DNA sequences of the present invention can also be used for the design of appropriate primers which hybridize with segments of the ataxia gene or fragments thereof under stringent conditions. Appropriate primer sequences can be constructed which are useful in the diagnosis of people who have a genetic defect causing ataxia. Such primers comprise nucleic acid sequences having a length of 10 – 40 nucleotides, preferably 15 – 30 nucleotides. Especially preferred primers are given in example 3.

In general, DNA sequences according to the present invention are understood to embrace also such DNA sequences which have at least 80 %, preferably at least 90 %, or at least 95 % or 98 % sequence identity to the specific DNA sequences according to the present invention. This also includes such nucleic acid sequences which are degenerate to the specific sequences shown, based on the degeneracy of the genetic code, or fragments thereof which hybridize under stringent conditions with the specifically shown DNA sequences. Hybridisation is to be understood to take place at high stringency conditions using Church buffer (0.5 M NaPi pH 7.2, 7% SDS, 1 mM EDTA) at 65°C and by washing in 40 mM NaPi, 1% SDS at 65°C.

In principle, all oligonucleotide primers and probes for amplifying and detecting a genetic defect responsible for ataxia in a biological sample are suitable for amplifying a target ataxia associated sequence. Especially, suitable exon specific primer pairs can be provided. Subsequently, a suitable detection, e.g. a radioactive or non-radioactive label is carried out.

Also, a single stranded RNA can be used as target. Methods for reversed transcribing RNA into cDNA are also well known and described in Sambrook et al., Molecular Cloning: A Laboratory Manual, New York, Cold Spring Harbor Laboratory 1989. Alternatively, preferred methods for reversed transcription utilize thermostable DNA polymerases having RT activity.

Further, the technique described before can be used for selecting those person from a group of persons being of short stature characterized by a genetic defect and which allows as a consequence a more specific medical treatment.

In another subject of the present invention, the ataxia protein can be used as pharmaceutical agents. These proteins initiate a still unknown cascade of biological effects on a molecular level involved with ataxia. They can be used in the treatment of ataxia, and related diseases based on the same etiology. Within the meaning of the present invention, the term "ataxia protein" also comprises proteins and functional fragments thereof, which have a similar or comparable physiological effect as the ataxia protein described in SEQ ID. No. 2. Such proteins comprise modifications, deletions, substitutions or variations of one or more amino acids, whereby the resulting variant has a homology of at least 80 %, preferably at least 90 %, 95 % or 99 % of the protein described in SEQ ID. 2. The ataxia proteins have a length of 300 – 600 amino acids, preferably 350 – 500 amino acids, and most preferably of 400 – 450 amino acids.

As used herein, the term „isolated" refers to the original derivation of the DNA molecule by cloning. It is to be understood however, that this term is not intended to be so limiting and, in fact, the present invention relates to both naturally occurring and synthetically prepared seqences, as will be understood by the skilled person in the art.

The DNA molecules of this invention may be used in forms of gene therapy involving the use of an expression plasmid prepared by incorporating an appropriate DNA sequence of this invention downstream from an expression promotor that effects

expression in a mammalian host cell. Suitable host cells are procaryotic or eucaryotic cells. Procaryotic host cells are, for example, E. coli, Bacillus subtilis, and the like. By transfecting host cells with replicons originating from species adaptable to the host, that is, plasmid vectors containing replication starting point and regulator sequences, these host cells can be transfected with the desired gene or cDNA. Such vectors are preferably those having a sequence that provides the transfected cells with a property (phenotype) by which they can be selected. For example, for E. coli hosts the strain E. coli K12 is typically used, and for the vector either pBR322 or pUC plasmids can be generally employed. Examples for suitable promotors for E. coli hosts are trp promotor, lac promotor or lpp promotor. If desired, secretion of the expression product through the cell membrane can be effected by connecting a DNA sequence coding for a signal peptide sequence at the 5′ upstream side of the gene. Eucaryotic host cells include cells derived from vertebrates or yeast etc.. As a vertebrate host cell, COS cells can be used (Cell, 1981; 23: 175 - 182), or CHO cells. Preferably, promotors can be used which are positioned 5′ upstream of the gene to be expressed and having RNA splicing positions, polyadenylation and transcription termination seqences.

The present invention is illustrated by the following examples.

Example 1
Patient

Patient T.G. is a ten year old boy suffering from slight mental retardation and severe cerebellar ataxia. In a MRI-scan severe hypo- and dysmyelinisation was observed infra- and supra- tentorial. Cytogenetically, the boy, as well as his healthy mother, exhibit an inversion of the X-chromosome with the breakpoints residing in Xp22 and Xq22, respectively.

Example 2
Identification of the ataxia gene

a) Florescence in situ hybridization (FISH)

FISH studies using appropriate cosmids were carried out according to published methods (Lichter and Cremer, 1992). In short, one microgram of the respective cosmid

clone was labeled with biotin and hybridized to human metaphase chromosomes under conditions that suppress signals from repetitive DNA sequences. Detection of the hybridization signal was via FITC-conjugated avidin. Images of FITC were taken by using a cooled charge coupled device camera system (Photometrics, Tucson, AZ).

b) Physical mapping

In order to investigate the breakpoint on the long arm of the X-chromosome, several YAC, PAC and cosmid clones (Vetrie et al., 1994) were chosen covering the region of CEPHy904G09223. DNA of these clones was used for FisH-analysis on metaphase spreads of the patient. It could be demonstrated that the PAC clone 1055C14 covers the breakpoint. The cosmids in close vicinity to the breakpoint-spanning PAC had already been sequenced by the Sanger Center in Cambridge.

c) Southern Blot Hybridisation

Southern blot hybridisations were carried out at high stringency conditions in Church buffer (0.5 M NaPi pH 7.2, 7% SDS, 1mM EDTA) at 65°C and washed in 40 mM NaPi, 1% SDS at 65°C.

d) FISH Analysis

Biotinylated cosmid DNA (insert size 32 - 45 kb) or cosmid fragments (10 - 16 kb) were hybridised to metaphase chromosomes from stimulated lymphocytes of patients under conditions as described previously (Lichter and Cremer, 1992). The hybridised probe was detected via avidin-conjugated FITC.

e) PCR Amplification

All PCRs were performed in 50 µl volumes containing 100 pg-200 ng template, 20 pmol of each primer, 200 µM dNTP's (Pharmacia), 1.5 mM $MgCl_2$, 75 mMTris/HCl pH9, 20mM $(NH_4)_2SO_4$ 0.01% (w/v) Tween20 and 2 U of Goldstar DNA Polymerase (Eurogentec). Thermal cycling was carried out in a Thermocycler GeneE (Techne).

f) Exon Amplification

For exon amplification experiments, the methods as previously described in Church et al., 1994 were used.

Example 3

Primer for the amplification of ataxia exons

Exons 2 and 3 as well as exons 4 and 5 were amplified in one PCR-reaction, as the introns are rather small.

GLRA4 Exon 1 For : 5'-ATG ACA ACT CTT GTT CCT GCA ACC CTC TCC-3'
GLRA4 Exon 1 Rev : 5'-TGG CTA GTG TTT GCA TGC ACC-3'
PCR conditions : 30'' 94°C, 30'' 58°C, 30'' 72°C, 38 cycles

GLRA4 Exon 2 For : 5'-GCA CAT AAC TGG CCT CAG ACT T-3'

GLRA4 Exon 3 Rev : 5'-CAC TCA ACA TAG GCT GGA AGT-3'
PCR conditions : 30'' 94°C, 30'' 60°C, 45'' 72°C, 38 cycles

GLRA4 Exon 4 For : 5'-CCT GAG ATG TGT TCC CAA CAT-3'

GLRA4 Exon 5 For : 5'-CCA GTA AGC CGA TGT CAC TTC-3'
GLRA4 Exon 5 Rev : 5'-GTT ATT CCA GGC TCT CTG TGA-3'
PCR conditions : 30'' 94°C, 30'' 59°C, 30'' 72°C, 38 cycles

GLRA4 Exon 6 For : 5'-CAG CAT CCA TAC TCT GCA GC-3'
GLRA4 Exon 6 Rev : 5'-AGG TTC TCC TGT GGC TCA CA-3'
PCR conditions : 30'' 94°C, 30'' 60°C, 30'' 72°C, 38 cycles

GLRA4 Exon 7 For : 5'-TCA GGC TCA GCT ACA GGC TG-3'
GLRA4 Exon 7 Rev : 5'-GGT ACT CTA TGG CAA GCA AGT T-3'
PCR conditions : 30'' 94°C, 30'' 58°C, 30'' 72°C, 38 cycles

GLRA4 Exon 8 For : 5'-GTG TCC TAC GTG AAG GCA AT-3'
GLRA4 Exon 8 Rev : 5'-TCC AAG CGT TGG CGC CTC T-3'
PCR conditions : 30'' 94°C, 30'' 58°C, 30'' 72°C, 38 cycles

GLRA4 Exon 9 For : 5'-CCA AGG CAC CTT GTC TGC ATA ACA-3'
GLRA4 Exon 9 Rev : 5'-GGA GAT GGT GTC AAT TCT CTT GGC-3'
PCR conditions : 30'' 94°C, 30'' 59°C, 30'' 72°C, 38 cycles

Claims

1. An isolated human nucleic acid sequence encoding a human ataxia protein having essentially the amino acid sequence of SEQ ID NO: 2.

2. A nucleic acid sequence according to claim 1 having the cDNA sequence according to SEQ ID NO: 1 or sequences substantially complementary to said cDNA sequence or fragments that are substantially complementary to fragments of said cDNA sequence.

3. A nucleic acid sequence according to claim 1 which is a genomic sequence.

4. A nucleic acid sequence according to claims 1 – 3 comprising the SEQ ID NO: 3.

5. A nucleic acid sequence according to claims 1 – 4 comprising the SEQ ID NO: 5.

6. A nucleic acid sequence according to claim 3 and comprising the SEQ ID NO: 6.

7. A recombinant host cell containing a nucleic acid sequence according to any of claims 1 – 6.

8. A vector comprising a nucleic acid sequence according to any of claims 1 – 6.

9. A protein encoded by any of the sequences according to claim 1 – 6 or a functional fragment or variant thereof.

10. A protein having the amino acid sequence selected from the group consisting of a) the amino acid sequence shown in SEQ ID NO. 2; b) a portion of the amino acid sequence shown in SEQ ID NO. 2; c) amino acid sequences which are homologous variants of the amino acid sequence shown in SEQ ID NO. 2; or d) portions of amino acid sequences which are homologous variants of the amino acid sequence shown in SEQ ID NO. 2.

11. A recombinant nucleic acid sequence comprising a genomic or cDNA sequence that encodes a protein according to claim 10.

12. A recombinant nucleic acid sequence according to claim 11 wherein said genomic DNA or cDNA sequence is operatively linked to an expression control sequence in said nucleic acid molecule.

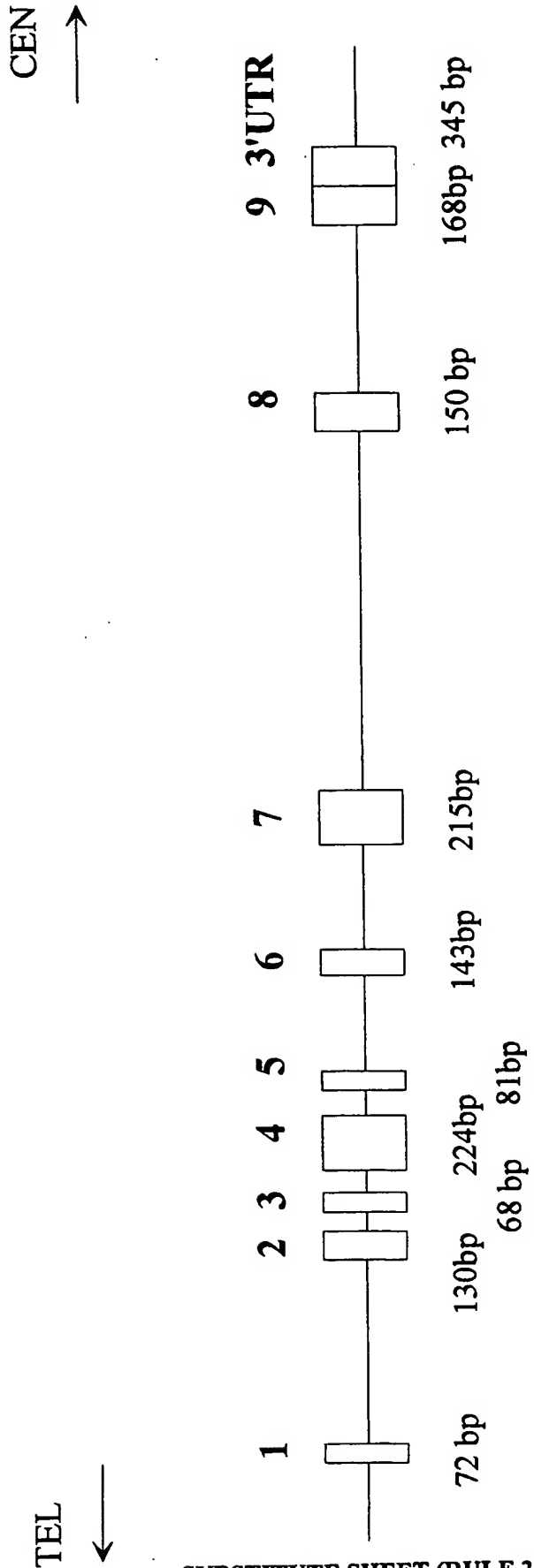
13. A method of detecting a genetic defect in the gene responsible for ataxia wherein the gene comprises an nucleic acid sequence according to claim 1, said method comprising the steps of a) amplifying one or more fragments of said gene and the ataxia gene known to be normal by the polymerase chain reaction (PCR) with the same or substantially the same PCR primers; b) determining whether said ataxia gene contains any mutations or genetic defects by comparing the PCR products of the amplification of said gene with those from the amplification of the normal gene, and c) detecting differences between the PCR products associated with mutations.

14. A method according to claim 13, wherein the gene comprises a nucleic acid sequence according to SEQ ID No. 3 (exon 8) or fragments thereof.

15. A method according to claim 13 or 14, wherein the gene comprises a nucleic acid sequence according to SEQ ID No. 5 (exon 9) or fragments thereof.

16. A method for identifying or screening of candidates for pharmaceutical agents useful for the treatment of disorders relating to mutations in the ataxia gene comprising providing a test system containing a transformed host cell according to claim 7 and determining variations in the phenotype of said cells or variations in the expression products of said cells after contacting said cells with said candidate pharmaceutical agents.

FIG. 1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aaa tct gga acc aag ggg tcc cag ccc atg tcc ccc tct gat ttc cta  144
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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 00/02600

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/705 C12Q1/68 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, BIOSIS, SCISEARCH, MEDLINE, EMBASE, EPO-Internal, BIOTECHNOLOGY ABS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MATZENBACH BERND ET AL: "Structural Analysis of Mouse Glycine Receptor alpha Subunit Genes: Identification and chromosomal localization of a novel variant, alpha-4." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 4, 1994, pages 2607-2612, XP002142164 ISSN: 0021-9258 abstract; figure 3 --- -/--	1,7-9, 11,13,16

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 July 2000	24/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gurdjian, D

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INTERNATIONAL SEARCH REPORT

Inter ›nal Application No
PCT/EP 00/02600

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	GRENNINGLOH G ET AL: "ALPHA SUBUNIT VARIANTS OF THE HUMAN GLYCINE RECEPTOR PRIMARY STRUCTURES FUNCTIONAL EXPRESSION AND CHROMOSOMAL LOCALIZATION OF THE CORRESPONDING GENES" EMBO (EUROPEAN MOLECULAR BIOLOGY ORGANIZATION) JOURNAL, vol. 9, no. 3, 1990, pages 771-776, XP002142165 ISSN: 0261-4189 abstract; figure 2 ---	1,7-9, 11,13,16
A	HILLIER L. ET AL.: " zu62b06.s1 Soares_testis_NHT Homo sapiens cDNA clone IMAGE:742547 3' similar to TR:G817957 G817957 GLYCINE RECEPTOR SUBUNIT ALPHA 4" EMBL DATABASE ; ACCESION NUMBER AA400068, 23 April 1997 (1997-04-23), XP002142166 the whole document ---	1,7-9, 11,13,16
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A	BECKER CM ET AL: "ISOFORM-SELECTIVE DEFICIT OF GLYCINE RECEPTORS IN THE MOUSE MUTANT SPASTIC" NEURON, 1992, 8, 283-289, XP002142168 abstract -----	1,7-9, 11,13,16

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